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A Fiber Optic Biosensor Based on Hydrogel-Immobilized Enzyme Complex for Continuous Determination of Cholesterol and Glucose

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Abstract
A multiparameter fiber optic biosensor for continuous determination of cholesterol and glucose was developed. This sensor was based on poly(*N*-isopropylacrylamide) (PNIPAAm)-immobilized glucose oxidase (GOx) complex (PIGC) and immobilized cholesterol oxidase (COD). The immobilized COD catalysis to the oxidation of cholesterol and PIGC catalysis to the oxidation of glucose could be performed at different temperatures. Therefore, the sensor could detect cholesterol and glucose continuously by changing temperature. The optimal detection conditions for glucose were achieved with pH 6.5, 30 °C, and 10 mg GOx (in 100-mg carrier), and those for cholesterol were achieved with pH 7.5, 33 °C, and 25 mg COD (in 250-mg carrier). The sensor has the cholesterol detection range of 20–250 mg/dL and the glucose detection range of 50–700 mg/dL. This biosensor has outstanding repeatability and selectivity, and the detection results of the practical samples are satisfactory.

Keywords PNIPAAm · Multiparameters · Fiber optic biosensor · Continuous detection · Phase delay

Introduction

Diabetes mellitus is a group of metabolic disorders causing the high glucose concentration in the blood, which afflicts millions of people in the world. Patients with diabetes are suffering from metabolic disorder, also exposed to significant long-term risks of complications, such as

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heart disease, cardiovascular disease, kidney failure, and blindness [1]. Cholesterol is an important biomarker for many diseases. The high levels of cholesterol in blood can cause the diseases including heart disease, hypertension, arteriosclerosis, coronary artery disease, and cerebral thrombosis [2]. It was reported that high glucose could lead to increased macrophage cholesterol accumulation in mice, and the large accumulation of cholesterol in macrophage was one of the main causes of atherosclerosis [3]. The results strongly suggested that high level of glucose in diabetes could increase the macrophage cholesterol accumulation and accelerate the risk of atherosclerosis. So, the development of fast-response, reliable, and low-cost method for continuous determination of glucose and cholesterol concentrations is important to clinical medicine and human health.

A variety of methods have been developed for glucose or cholesterol assays, including HPLC [4–7], electrode [2, 8–10], electrochemical sensor [11–14], and optical sensing [15–19]. Q. L. Huang et al. [20] developed a dual enzymatic biosensor for simultaneous determination of glucose and cholesterol in serum and peritoneal macrophages of diabetic mice. Fiber optic biosensors have many advantages such as high sensitivity, fast response, and immunity from electrical interference [21], and can be the effective ways to detect cholesterol and glucose.

Thermo-responsive hydrogels with tunable network structures [22] have the capability to respond to external temperature changes [23]. This characteristic makes them especially useful for the temperature-controlled release of macromolecular drugs [24, 25]. Poly(*N*-isopropylacrylamide) (PNIPAAm) is a well-known temperature-sensitive polymer and demonstrates a lower critical solution temperature (LCST) of ~ 32 °C in aqueous solution [26]. If PNIPAAm is used to complex with glucose oxidase (GOx), the enzyme performance can be manipulated by changing the temperature. When the temperature is higher than its LCST, the hydrogel will shrink and the capsulated GOx is isolated from the substrate, which will not perform the enzymatic catalysis. On the other hand, when the temperature is lower than its LCST, the hydrogel will swell, which allows the enzyme to contact with substrate and cause enzymatic catalysis reaction to take place. Therefore, the complex will have "on-off" effect for the GOx catalysis to glucose oxidation. Using this and other immobilized enzymes (such as immobilized COD) and controlling the detection temperature, it will be possible to develop the multiparameter biosensor and detect different species such as cholesterol and glucose at different temperatures.

In our previous work, PNIPAAm was combined with the immobilized GOx to form PNIPAAm-immobilized GOx complex (PIGC) with in situ complex method and a temperature-controlling fiber optic glucose sensor based on PIGC was developed [27]. In this work, GOx and cholesterol oxidase (COD) are immobilized on SiO_2 nanoparticles, respectively. Using optical oxygen-sensing film [28] and lock-in technology [29], a fiber optic multiparameter biosensor based on PIGC and immobilized COD is fabricated. The sensor can perform the continuous determination of cholesterol and glucose concentrations at different temperatures effectively, indicating a promising prospect of practical application. To the best of our knowledge, this multiparameter fiber optic biosensor based on PIGC and immobilized COD has never been reported before.

Experimental

Reagents and Chemicals

GOx (E.C. 1.1.3.4, 100 U/mg) was obtained from *Aspergillus niger*. COD with a specific activity of more than 10 U/mg was purchased from Aldrich-Sigma. Glucose, cholesterol, and

$Ru(bpy)_3Cl_2$ (99.0%) were obtained from Aldrich-Sigma. All reagents were with analytical grade and used without further purification. Double-distilled water was used throughout the experiments. The optical oxygen-sensing membrane was prepared as previously described [28]. PNIPAAm-immobilized GOx complex (PIGC) was prepared using our reported method [27]. The immobilized COD was prepared using the method similar to that in our previous work [30].

Apparatus

A lock-in amplifier (SR830, Stanford Research System, USA) was used for measuring the phase delay of sensor head.

Preparation and Principle of Fiber Optic Multiparameter Biosensor

The detecting system is similar to that in our previous work [27]. It consists of a lock-in amplifier, a LED with the excitation wavelength of 416 nm as the light source, a sensor head with an oxygen-sensing membrane, a temperature controller, and a computer for data processing (see Fig. 1).

This sensor was based on the fluorescence quenching and consumption of oxygen. The oxidation of cholesterol and glucose catalyzed by COD and GOx, respectively, would consume oxygen in the solution. Using PIGC and raising the temperature at above the LCST of PNIPAAm, the cholesterol could be oxidized first with the catalysis of COD while PIGC would not catalyze the oxidation of glucose. After the completion of cholesterol oxidation, the temperature was lowered to that below the LCST and the oxidation of glucose occurred with

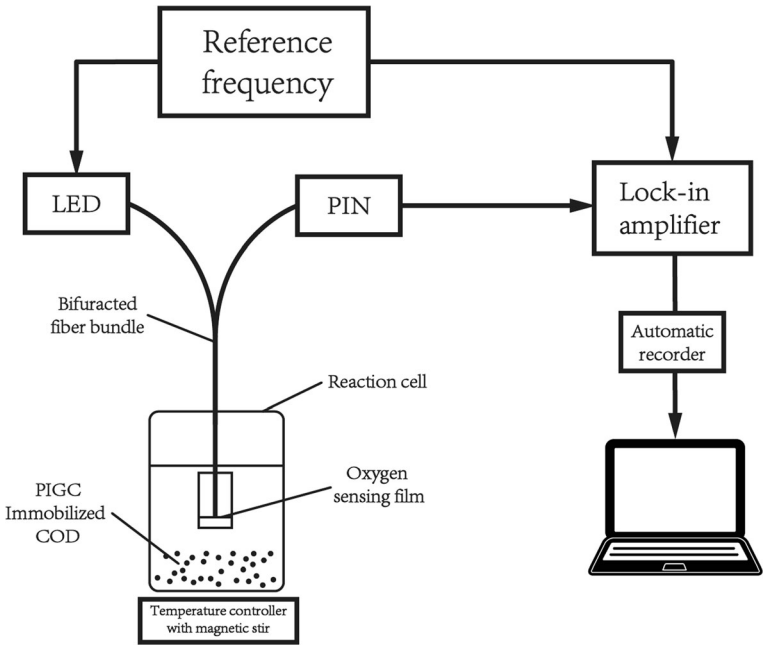

Fig. 1 Schematic diagram of the detecting system

the catalysis of PIGC. Both of the two oxidation processes would consume oxygen. Therefore, at the temperature above LCST, the immobilized COD and optical oxygen-sensing membrane acted as the cholesterol sensor. At the temperature below LCST, PIGC and optical oxygen-sensing membrane acted as the glucose sensor. Therefore, we had fiber optic cholesterol sensor and fiber optic glucose sensor with the same detecting system. By detecting the fluorescence of $Ru(bpy)_3Cl_2$ quenched by oxygen, the oxygen concentration changes in these two oxidation processes were determined respectively. Since a lock-in amplifier was used, the quenching could be described as the phase delay difference φ [27]. By detecting the data of phase delay difference φ in these two oxidation processes, the quantification of glucose and cholesterol was achieved.

Measurements

To detect the cholesterol and glucose concentrations at different temperatures, measurements were performed with the setup shown schematically in Fig. 1. The sensor head was placed into a tiny reaction cell which contained PIGC, immobilized COD, and cholesterol and glucose buffer solution. An entire airtight reaction cell was introduced to eliminate the interference of oxygen from the open air. A temperature controller was used to control the temperature of the reaction cell. At first, the temperature was raised to 36 °C (above LCST) and the phase delay difference φ was recorded for the detection of cholesterol concentration. Then, the temperature was lowered to 25 °C (below LCST) and φ was recorded for the detection of glucose concentration. All the measurements were performed in triplicate. After a simple washing of the sensor head, PIGC, and immobilized COD, the following measurement could be performed.

Results and Discussions

Influence Factors to the Phase Delay Difference of the Sensor

For the fiber optic cholesterol sensor, φ was defined as the difference between the phase delay of the sensor head with cholesterol concentration of 100 mg/dL and with no cholesterol in the solution.

The pH effect on the fiber optic cholesterol sensor was investigated from pH 5.0 to 9.0. φ increases from pH 5.0 to 7.5 and reaches its maximum value at pH 7.5. After pH 7.5, φ decreases (Fig. 2a). Therefore, the optimal pH for this sensor is pH 7.5.

The temperature effect on the fiber optic cholesterol sensor was studied from 10 to 38 °C. The results indicate that with the increase of temperature, φ increases when the temperature is below 33 °C and decreases slowly when the temperature is higher than 33 °C (Fig. 2b). So, 33 °C is the optimal temperature. From Fig. 2b, we can see that the sensor response is satisfactory at 36 °C, so we can detect cholesterol at 36 °C.

Different amounts of COD (immobilized in 10 times carriers) were used to investigate the influence of immobilized COD amount on the sensor performance. The results in Fig. 2c indicate that φ increases with the increase of COD amount from 5 to 25 mg. When the COD amount is higher than 25 mg, φ decreases. So, 25 mg is selected as the optimal COD amount.

For the fiber optic glucose sensor, φ was defined as the difference between the phase delay of the sensor head with glucose concentration of 200 mg/dL and with no glucose in the

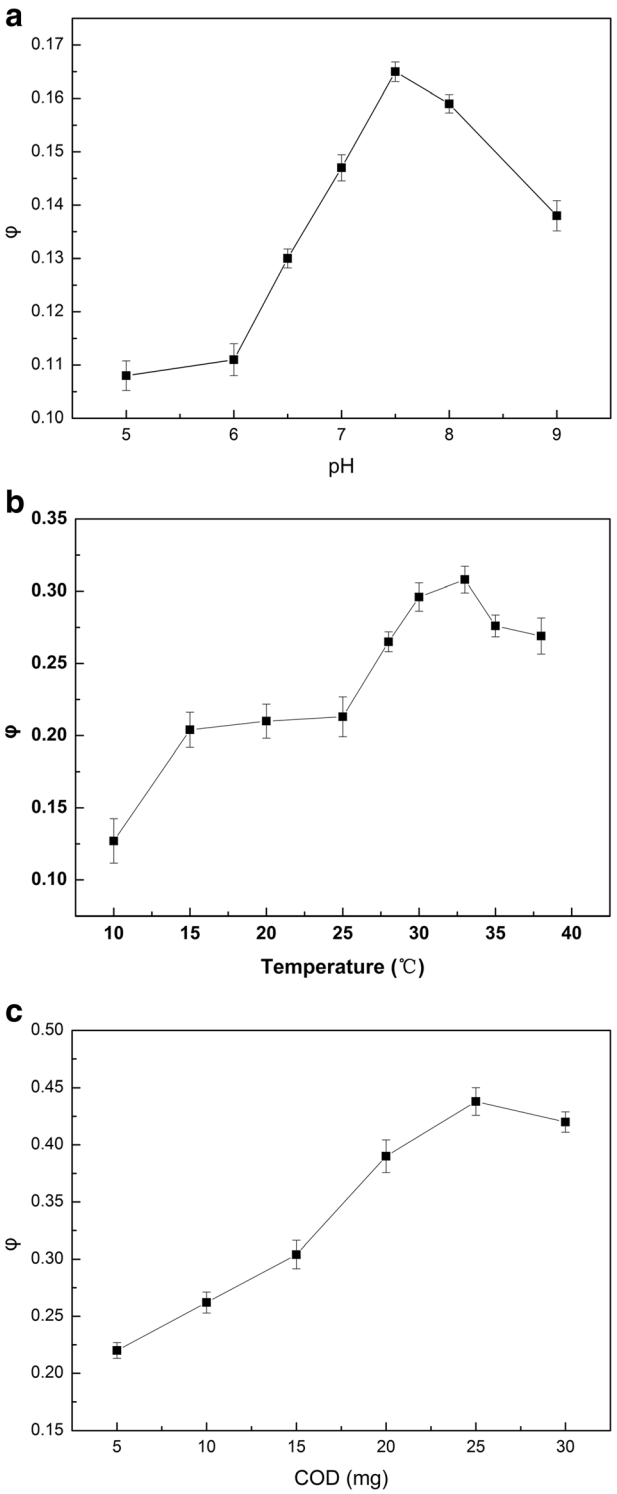

Fig. 2 The effect of pH (**a**), temperature (**b**), and COD amount (**c**) on the response of the fiber optic cholesterol sensor

solution. The pH, temperature, and GOx amount effects on φ of the glucose sensor were studied in our previous work, and the optimal pH, temperature, and GOx amount were achieved with pH 6.5, 30 °C, and 10 mg (in 100-mg carrier) [27].

Continuous Determination of Cholesterol and Glucose with the Sensor

For the continuous determination of cholesterol and glucose by using this sensor, cholesterol was determined first, so it was necessary to know the influence of glucose on the determination of cholesterol in the mixed solution. The results in Fig. 3 show that the glucose concentrations in the range from 100 to 600 mg/dL have no significant influence on the determination of cholesterol (RSD = 3.56%). The sensor performed the continuous determination of cholesterol and glucose at different temperatures. When the temperature was 36 °C (above LCST), PNIPAAm shrank and the capsulated GOx was isolated from the substrate; PIGC had no catalysis effect on the oxidation of glucose. The immobilized COD catalyzed the oxidation of cholesterol and the sensor performed the determination of cholesterol. With 100 mg/dL of glucose concentration in the mixed solution, a typical response curve with the phase delay difference φ of the sensor head and the cholesterol concentration can be observed (Fig. 4). There is a good linear relationship between φ and the cholesterol concentration in the range of 20–250 mg/dL. The linear graph is defined by the equation of $Y = 0.00152X + 0.1557$ and $R^2 = 0.9976$. The response time was taken as 200 s and the detection limit was 4.2 mg/dL ($S/N = 3$).

After the completion of cholesterol oxidation, the temperature was lowered to 25 °C (below LCST). PIGC had outstanding catalysis effect on the oxidation of glucose and the sensor carried out the determination of glucose. A good linear relationship between φ and the glucose concentration in the range of 50–700 mg/dL can be observed (Fig. 5). The linear graph is defined by the equation of $y = 0.000596x + 0.00641$ and $R^2 = 0.9936$. The response time was taken as 200 s and the detection limit was 11.4 mg/dL ($S/N = 3$). Because our sensor is based

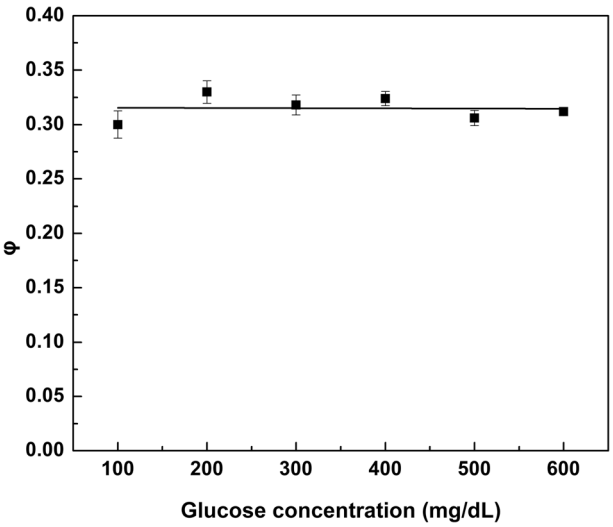

Fig. 3 The influence of glucose concentration on the determination of cholesterol. pH = 7.5. $T = 36$ °C. COD amount was 25 mg (in 250-mg carrier). Cholesterol concentration was 100 mg/dL

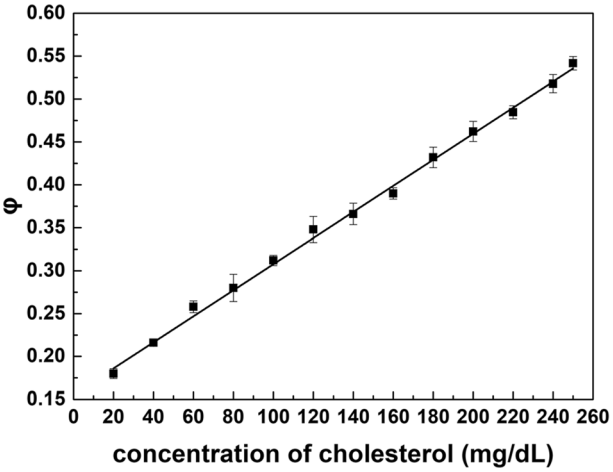

Fig. 4 Response curve at various concentrations of cholesterol at 36 °C. pH = 7.5. T = 36 °C. COD amount was 25 mg (in 250-mg carrier). Glucose concentration was 100 mg/dL

on oxygen consumption, in high-altitude areas, the dissolved oxygen concentration in water solution might be slightly lower than that in normal areas, so the detection range of the sensor might decrease slightly.

Repeatability, Lifetime, and Selectivity of the Sensor

Repeatability is another important factor for a biosensor. φ of the sensor head was investigated when it was alternately exposed to 200 mg/dL glucose solution and PBS solution with no glucose three times. The glucose biosensor exhibited a desirable repeatability (n = 3, RSD = 2.83%). The similar experiments were performed for 100 mg/dL cholesterol and the repeatability was also satisfactory (n = 3, RSD = 3.13%).

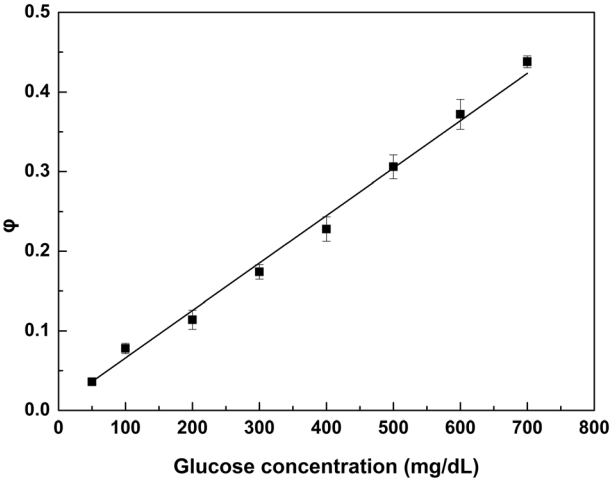

Fig. 5 Response curve at various concentrations of glucose at 25 °C. pH = 6.5. T = 25 °C. GOD amount was 10 mg (in 100-mg carrier)

Table 1 Interference test of the fiber optic glucose sensor on exposure to various interferents

Interference term	[S] (mg/dL)	Interference rate (%)* $(C - C_0)/C_0 \times 100\%$
Vitamin C (cholesterol)	25.5	− 4.15
Vitamin C (glucose)	25.5	2.34
Uric acid (cholesterol)	242.1	− 1.76
Uric acid (glucose)	242.1	− 2.61
Dopamine (cholesterol)	10.5	− 1.47
Dopamine (glucose)	10.5	1.78
Urea (cholesterol)	249.0	2.94
Urea (glucose)	249.0	− 3.33
Lactose (cholesterol)	20.0	− 4.41
Lactose (glucose)	20.0	3.57

*C is the detected concentration and C_0 is the actual concentration

In order to investigate the lifetime of the sensor, the response values of the oxygen-sensing film in contact with 100 mg/dL glucose solution and 100 mg/dL cholesterol solution were recorded after keeping the film in water for 7 days. The results showed that the change of phase delay of the sensor under optimal conditions only decreases by 8.6% and 8.3%, respectively. The indicator leaking from the film might be one reason for the loss of sensitivity. After being stored for 1 month at 4 °C, the immobilized GOx and immobilized COD maintained 84% and 60% of its initial activity, showing a good stability for the immobilized enzymes.

The actual concentration of cholesterol and glucose was selected as 100 mg/dL, and the interference rates in the presence of vitamin C, uric acid, dopamine, urea, and lactose were used as indicators for the sensor selectivity. From the results in Table 1, we can conclude that all of them do not cause significant interference on the determination of cholesterol and glucose, indicating a good selectivity of this sensor.

Real Sample Detection

The recovery test was performed using human serum as the practical sample. The test was carried out under four different added cholesterol concentrations (the glucose concentration of 200 mg/dL was added in each sample) (Table 2). All the tests were performed in triplicate measurements. The results in Table 2 indicate that this sensor is useful in the determination of cholesterol in practical samples.

Table 2 Determination of cholesterol in human serum using the proposed sensor

Sample	Cholesterol added (mg/dL)	Cholesterol detected (mg/dL)	Recovery* (%)
1	0	116.22	
2	40	154.45 ± 2.68	95.58
3	80	198.16 ± 2.36	102.43
4	120	231.38 ± 2.12	95.97

*Recovery is the ratio of the detected cholesterol concentration to the added cholesterol concentration in practical samples

Table 3 Determination of glucose in human serum using the proposed sensor

Sample	Glucose added (mg/dL)	Glucose detected (mg/dL)	Recovery* (%)
1	0	84.12 ± 2.35	
2	50	131.89 ± 2.89	95.54
3	100	187.25 ± 2.52	103.13
4	200	275.96 ± 2.38	95.92
5	300	380.73 ± 1.94	98.87
6	500	567.87 ± 1.73	96.75

The recovery test of glucose was performed under six different added glucose concentrations in human serum (the cholesterol concentration of 100 mg/dL was added in each sample) (Table 3). All the tests were performed in triplicate measurements. The results in Table 3 show that the determination of glucose in practical samples using this sensor is satisfactory.

We compare our sensor with other reported methods for cholesterol and glucose detection. The results shown in Table 4 indicate that for the glucose detection our sensor has good selectivity, repeatability and recovery rate, and satisfactory detection range. The results in Table 5 show that for the cholesterol detection, our sensor also has satisfactory properties. For the healthy people, the glucose and cholesterol ranges in blood are 3.9–6.1 mmol/L and 2.86–5.98 mmol/L, respectively. Therefore, our sensor has potential application prospect.

Table 4 Comparison of the proposed sensor with other glucose detection methods

Method	Detection range (mmol/L)	Selectivity	Repeatability (RSD(%))	Recovery rate (%)	Ref.
HPLC	0.0056–0.56	–	2.56	101–103	[31]
Quantum dots	0.005–0.4	Good	3.1	95.5–108.9	[32]
Electrochemical	1.0×10^{-5} to 80	Good	4.60	90.1–104.8	[33]
Chemiluminescence	8.5×10^{-4} to 0.1	Good	2.9	93.8–110.1	[34]
Spectroscopic	5.5×10^{-4} to 3.2×10^{-2}	Good	1.9	–	[35]
Proposed sensor	2.78–38.85	Good	2.83	95.54–103.13	This work

Table 5 Comparison of the proposed sensor with other cholesterol detection methods

Method	Detection range (mmol/L)	Selectivity	Repeatability (RSD(%))	Recovery rate (%)	Ref.
HPLC	2.6×10^{-5} to 5.2×10^{-4}	–	4.10	94.3–102.3	[7]
Quantum dots	2.6×10^{-3} to 6.5×10^{-2}	Good	0.9	103–110	[36]
Electrochemical	0.0025–0.0275	Good	4.17	96.7–101.0	[37]
Chemiluminescence	0.0033–1.0	Good	4.6	97.8–106.5	[38]
Spectroscopic	0.002–0.05	Good	< 7.4	90.0–104.4	[39]
Proposed sensor	0.52–6.47	Good	3.13	95.58–102.43	This work

Conclusions

Based on PIGC and immobilized COD, a fiber optic biosensor was developed for continuous determination of cholesterol and glucose by changing the temperature. The optimal detection conditions for glucose and cholesterol were achieved. At 36 °C, there is a good linear relationship between φ and cholesterol concentration in the range of 20–250 mg/dL. At 25 °C, a good linear relationship between φ and glucose concentration was observed in the range of 50–700 mg/dL. This biosensor has good repeatability and selectivity and can be used for the determination of practical samples, showing a promising prospect of practical application.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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